Binding of [(dien)PtCl] Cl to poly(dG-dC).poly(dG-dC) facilitates the B→Z conformational transition

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ABSTRACT

Chlorodiethylenetriamineplatinum(II) chloride, [(dien)PtCℓ]Cℓ, bound to ≤ 10% of the nucleotide bases of poly(dG-dC)•poly(dG-dC) reduces the amount of ethanol necessary to bring about the B→Z conformational transition in proportion to the amount of platinum complex bound as monitored by CD spectroscopy. The transition may be effected by 25% ethanol with 9.3% of the bases modified and by 36% ethanol with 5.4% of the bases modified. With an unmodified polymer an ethanol concentration of 55-60% is necessary to bring about the transition. The assignment of the Z conformation was supported by ^{31}P NMR spectroscopy. This covalent modification of the DNA is reversed by treatment with cyanide ion after which the normal amount of ethanol is necessary to achieve the transition. The platinum complex shows no enhanced binding to DNA in the Z versus the B conformation. Between 20 and 33% (saturation binding) modification, [(dien)PtCℓ]Cℓ binds cooperatively to the heterocopolymer as judged by CD spectroscopy. At this high level of modification it is no longer possible to induce the Z DNA structure with ethanol. When [(dien)PtCℓ]Cℓ is bound to preformed (with ethanol) Z DNA at saturating levels the CD spectrum is altered but reverts to the spectrum of highly modified DNA upon removal of ethanol. The antitumor drug cis-diamminedichloroplatinum(II), cis-DDP, binds to poly(dG-dC)•poly(dG-dC) and alters the CD spectrum. It does not facilitate the B→Z conformational change, however, and actually prevents it from happening even at very high ethanol concentrations.

INTRODUCTION

In high concentrations of salt or ethanol poly(dG-dC)•poly(dG-dC) adopts an alternate conformation (1,2) which is characterized by an almost complete inversion of the circular dichroism (CD) spectrum of the low salt conformation. The presence of an additional peak in the ^{31}P NMR spectrum (3,4,5) and changes in the Raman spectrum (6,7) are also observed. These spectral changes have been attributed to the formation of a left handed helical structure classified as Z DNA and exemplified by the crystal structure of the self complementary hexanucleoside pentaphosphate d(CpGpCpGpCpG) (8,9). DNA in the Z form is characterized by a

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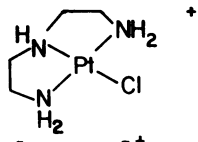
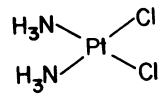
left handed helical sense and a dinucleotide repeat unit resulting in 12 base pairs per helical turn. The dC sugars have C2' endo and the internal dG sugars have C3' endo ring puckering. Deoxycytidine is in the anti conformation while deoxyguanosine is in the syn conformation. As a direct consequence of deoxyguanosine being in the syn conformation the N7 and C8 positions are on the outside of the helix and exposed to solvent. This enhanced exposure of N7 and C8 is particularly relevant with respect to the binding of carcinogens, drugs, and metals to the DNA.

We reported previously that poly(dG-dC)•poly(dG-dC) modified with N-2-acetylaminofluorene (AAF) at 28% of its bases at the C8 position of guanine exhibits a circular dichroism spectrum characteristic of the Z conformation (10). Moreover, poly(dG-dC)•poly(dG-dC) modified by AAF at 3% of its bases exhibited a CD spectrum characteristic of B form DNA but could be transformed to the Z conformation at a lower concentration of ethanol than is necessary with the unmodified polymer. The ability of bound AAF and N-2-aminofluorene to facilitate the B→Z transition at lower than normal ethanol concentrations has also been reported by other workers (11). Methylation at the N7 position of deoxyguanosine (12) and at the C5 position of deoxycytidine (13) in poly(dG-dC)•poly(dG-dC) also facilitates the B→Z transition. The antibiotic mitomycin-C which binds covalently to DNA induces a conformational change in poly(dG-dC)•poly-(dG-dC) characteristic of Z DNA (14).

Our studies of the mechanism of action of the antitumor drug cis-diamminedichloroplatinum(II), cis-DDP, and, in general, of the effects of metal binding on DNA structure (15, 16) led us to investigate the interactions of cis-DDP and chlorodiethylenetriamineplatinum(II) chloride, [(dien)PtCl]Cl, with poly(dG-dC)•poly(dG-dC).



[(dien)PtCl]$^+$



cis-DDP

The platinum complexes seemed to be good candidates for inducing the B→Z transition in poly(dG-dC)•poly(dG-dC) because N7 of deoxyguanosine, a primary site of metal binding to DNA (16), lies on the outside of the Z DNA helix. Moreover, since covalent Pt-DNA linkages can be reversed by addition of cyanide ion (17) we were interested to learn whether

platinum complexes would facilitate the B→Z transition in a reversible manner.

As described in a preliminary report (18), [(dien)PtCℓ]Cℓ was found to promote the formation of Z form poly(dG-dC)•poly(dG-dC) in aqueous ethanol. Here we describe our results in detail and present ^{31}P NMR evidence to support the assignment of the Z conformation. We also show that there is no preferential binding of this reagent to poly(dG-dC)•poly(dG-dC) in the Z versus the B conformation. While this manuscript was in preparation Malfoy and coworkers published CD data showing that [(dien)PtCℓ]Cℓ induces the Z conformation in poly(dG-dC)•poly(dG-dC)(19).

MATERIALS AND METHODS

Poly(dG-dC)•poly(dG-dC) and poly(dG)•poly(dC) were purchased from P-L Biochemicals, Milwaukee, WI., USA. cis-DDP was prepared by a literature method (20). The compound [(dien)PtCℓ]Cℓ was prepared by allowing one equivalent of dien•3HCℓ to react with one equivalent of $K_2PtCℓ_4$ and adjusting the pH to 7. The white product was purified by fractional recrystallization from 0.1 N HCℓ. Analysis for platinum by atomic absorption spectroscopy and comparison of the infrared spectrum with the published spectrum for this compound (21) established its identity. The aquo form of [(dien)PtCℓ]Cℓ was prepared by allowing the complex to react at 37 °C for 12 h in the dark with 1.95 equivalents of $AgNO_3$. The reagent $[Co(NH_3)_6]Cℓ_3$ was purchased from Aldrich and used without further purification.

Reactions of the platinum complexes with the biopolymers were carried out in 1 mM sodium phosphate, 3-5 mM sodium chloride, pH 7.4 at 37 °C for 24 h. Reactions were quenched by raising the NaCℓ concentration to 0.2 M followed by freezing. Samples were later thawed and dialyzed against the above buffer in order to remove any unreacted platinum complex and excess salt. When the aquated form of [(dien)-PtCℓ]Cℓ was used, 5 mM $NaCℓO_4$ was substituted for NaCℓ in the reaction buffer. Reversal of platinum binding was achieved by incubation for 3 h at 37 °C in 0.2 M potassium cyanide followed by extensive dialysis. Platinum binding was quantitated on a Varian AA-375 atomic absorption spectrometer equipped with a carbon rod atomizer. Binding values are reported as r_b, the number of platinum atoms bound per nucleotide.

Circular dichroism studies were performed on a Cary model 60 or a Jasco model J40 spectropolarimeter. Samples were 0.05-0.1 mM in nucleo-

tide. Molar ellipticities, [θ], were calculated per nucleotide residue.

Poly(dG-dC)•poly(dG-dC) was sonicated with a Branson sonifier using a micro tip to an average length of 320 ± 100 base pairs as judged by polyacrylamide gel electrophoresis using the HinfI digestion fragments of pBR322 DNA for sizing. NMR spectra of approximately 70 A_{260} units of DNA dissolved in 6 mL of 10 mM cacodylate, 1mM EDTA, pD 7.6 buffer were obtained using a coaxial cylindrical insert (Wilmad) in order to insure that all the sample was within the receiver coil. Chemical shifts are reported relative to trimethylphosphate, uncorrected for the presence of ethanol. NMR spectra were recorded on a Bruker WM-300 spectrometer interfaced with an Aspect-2000 data system using a 20 mm broad band probe tuned to the ^{31}P resonance frequency of 121.513 MHz (7.05 Tesla field). 16K spectra were obtained using a pulse width of 75 μsec (70° tip angle) with a repetition time of 4.65 seconds. ^{1}H decoupling was achieved by broad band irradiation with 3 watts of power.

RESULTS AND DISCUSSION

Platinum Binding to Poly(dG-dC)•Poly(dG-dC). When the formal ratio (r_f) of [(dien)PtCℓ]Cℓ to nucleotide is between 0.05 and 0.3 approximately 80-90% of the platinum is bound to poly(dG-dC)•poly(dG-dC) after 24 h incubation at 37 °C. At an r_f greater than 0.3 there is a saturation binding level of approximately 0.33 platinum atoms per nucleotide. This value is larger than that of 0.25 observed (22) for the saturation binding of [(dien)PtCℓ]Cℓ to salmon sperm DNA and is presumably due to the greater fraction of G-C base pairs in poly(dG-dC)•poly(dG-dC).

In order to monitor conformational changes that occur upon binding of platinum complexes to poly(dG-dC)•poly(dG-dC) the circular dichroism spectra were measured. Figure 1 shows the CD spectra of poly(dG-dC)•poly(dG-dC) modified at various levels with [(dien)PtCℓ]Cℓ. With increasing binding the negative band at 250 nm is reduced in intensity and eventually disappears. At r_b = 0.11 the positive band at 275 nm is maintained but at higher binding levels it shifts to 295 nm and increases in intensity. For the sample with r_b = 0.275, there is a sixfold intensity enhancement. The change in molar ellipticity at 295 nm is plotted as a function of [(dien)PtCℓ]Cℓ binding in Figure 2. At r_b values greater than 0.2 there is a sharp cooperative increase in intensity which appears to level off at r_b = 0.3, the point of saturation binding. There is, however, no inversion of the CD spectrum

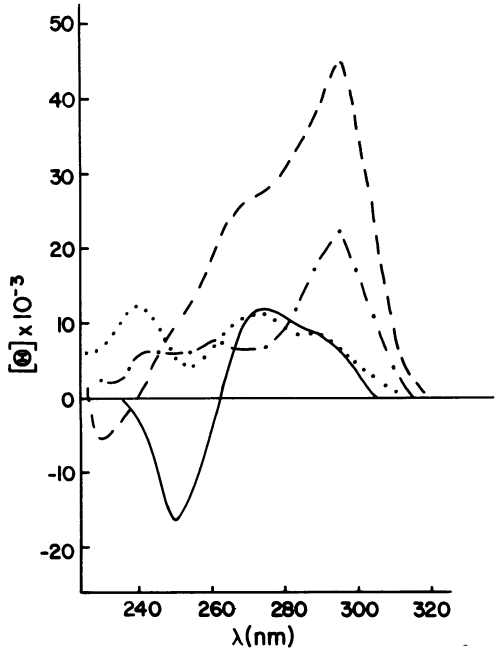


Figure 1. Circular dichroism (CD) spectra in 1 mM sodium phosphate, 5 mM NaCl, pH 7.4 buffer of poly(dG-dC)•poly(dG-dC) modified at the indicated binding levels with [(dien)PtCl]Cl. ——, r_b = 0; •••, r_b = 0.11; -•-, r_b = 0.23; - - -, r_b = 0.275.

characteristic of left-handed Z DNA.

The fact that [(dien)PtCl]Cl shows a saturation binding level of one platinum atom per three nucleotides accompanied by a cooperative increase in $[\theta]_{295}$ suggests that the modified poly(dG-dC)•poly(dG-dC) has a highly regular structure. One possibility consistent with the binding stoichiometry is shown below. Fiber X-ray diffraction or

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 Pt       Pt  Pt       Pt
 |        |   |        |
-G-C-G-C-G-C-G-C-G-C-G-C-
-C-G-C-G-C-G-C-G-C-G-C-G-
   |   |        |   |
   Pt  Pt       Pt  Pt
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solution low angle X-ray scattering experiments would provide an experimental test of this or other models. Platinum metallointercalation

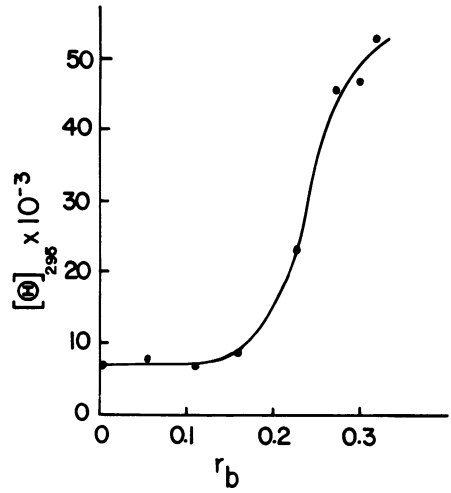

Figure 2. A plot of the molar ellipticity, [θ], at 295 nm versus r_b for samples of poly(dG-dC)•poly(dG-dC) modified with [(dien)PtCℓ]Cℓ.

reagents bound to DNA in a nearest neighbor excluded fashion give characteristic X-ray fiber diffraction patterns indicative of the platinum spacing along the duplex axis (15). The regular sequence of the heterocopolymer is probably a significant factor influencing the structure of poly(dG-dC)•poly(dG-dC) containing [(dien)PtCℓ]Cℓ at r_b = 1/3, since this platinum complex binds to salmon sperm DNA with no significant change in the 280 nm CD band. The diethylenetriamine ligand might also be a factor. Metal ions in the presence of polyamines (23) and metal complexes such as $[Co(NH_3)_6]^{3+}$ or $[Co(en)_3]^{3+}$ (24), en = ethylenediamine, induce compacted DNA structures that often exhibit characteristic, intense CD spectral bands. Thus hydrogen bonding interactions between the amino protons of the deoxyguanosine-bound $[Pt(dien)]^{2+}$ dication and the phosphate groups of the same or an adjacent duplex could stabilize the Pt-DNA structure.

The CD spectral changes that occur upon modifying poly(dG-dC)•poly(dG-dC) with cis-DDP are shown in Figure 3. With increasing levels of platinum binding there is an increase in the intensity of the 270 nm positive band and a decrease in intensity of the negative band at 250 nm. At an r_b value greater than 0.1 this effect is reversed with the band at 270 nm decreasing in intensity and becoming negative at r_b = 0.35. This initial increase in intensity of the 270 nm band with

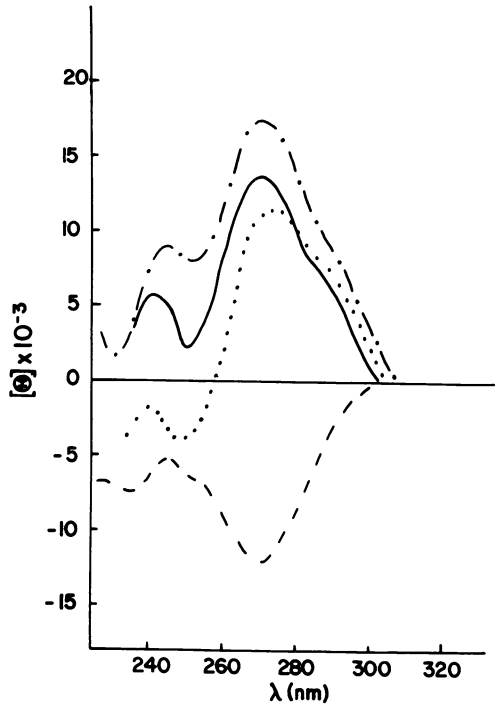

Figure 3. CD spectra in 1 mM sodium phosphate, 5 mM NaCl, pH 7.4 buffer of poly(dG-dC)•poly(dG-dC) modified with cis-diamminedichloroplatinum(II), DDP, at the indicated binding levels. •••, r_b = 0.06; -•-, r_b = 0.078; ——, r_b = 0.10; - - -, r_b = 0.35.

maximization at r_b = 0.1 followed by a decrease in intensity has been observed previously for the binding of cis-DDP to calf thymus (25), M. luteus (25), and salmon sperm (22) DNAs. The Pt-DNA structures that give rise to these characteristic CD spectra clearly must differ from that of [(dien)PtCl]Cl with poly(dG-dC)•poly(dG-dC). Although the CD spectrum of the sample having r_b = 0.35 is inverted, it is not the spectrum characteristic of Z DNA. The wavelength minimum occurs at 270 nm, compared with 295 nm for Z DNA, and the spectrum lacks the positive band at 260 nm. The cis-DDP induced conformational changes of DNA, monitored by circular dichroism studies, have previously been classified as Ψ(-) (23).

Figure 4 shows the CD spectra of samples of poly(dG-dC)•poly(dG-dC) modified with [(dien)PtCl]Cl at r_b values of 0.054 and 0.093. In the absence of ethanol both samples exhibit B DNA type spectra. Upon the

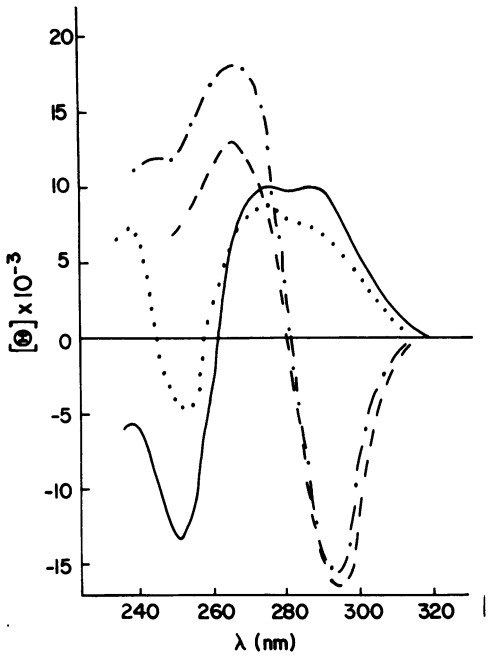

Figure 4. CD spectra in 1 mM sodium phosphate, 5 mM NaCl, pH 7.4 buffer of poly(dG-dC)•poly(dG-dC) modified to r_b values of 0.054 and 0.093 before (—— and •••, respectively) and after (- - -, 36% ethanol and -•-, 25% ethanol, respectively) the addition of the amount of ethanol necessary to induce the B→Z transition.

addition of ethanol they undergo a spectral transition at ethanol concentrations below the normally required amount of 55-60%. Since the new spectral parameters are identical to those observed with unplatinated DNA at high ethanol concentrations, we conclude that the [(dien)PtCl]Cl facilitated conformational change is that of B→Z DNA. The concentration of ethanol necessary to achieve the transition is inversely proportional to the level of platinum binding. The r_b = 0.054 sample required 36% ethanol and, at r_b = 0.093, 25% ethanol was needed. Interestingly, samples with r_b greater than 0.1 which exhibited the large positive CD at 295 nm showed no change in CD with the addition of ethanol and could never be forced into the Z conformation. The regular structure discussed above for this form of the modified polymer must therefore be sufficiently stable to resist the induction of Z DNA.

In our buffer, [(dien)PtCl]Cl was never able to induce the B→Z transition without added ethanol. Malfoy et al (19) show that in 1 mM

phosphate with no added NaCℓ poly(dG-dC)•poly(dG-dC) with [(dien)PtCℓ]Cℓ at an r_b of 0.12 is in the Z conformation. The reason that we do not observe the Z conformation in the absence of ethanol is due to the presence of 5 mM NaCℓ in our solutions. Malfoy et al (19) showed that with [(dien)PtCℓ]Cℓ bound to poly(dG-dC)•poly(dG-dC) there are two forms of Z DNA: a high salt form which is identical to Z DNA induced with high salt in the absence of any bound platinum complex and a low salt form which has slightly different CD spectral characteristics. At an intermediate salt concentration, approximately in the region where we worked, the CD spectrum of poly(dG-dC)•poly(dG-dC) with [(dien)PtCℓ]Cℓ bound at an r_b of 0.12 is close to that of the standard B form DNA. Dialysis of our samples against buffer containing no NaCℓ inverted the CD spectrum as expected (19).

In order to obtain independent evidence that the conformation of the polymer is switched to Z DNA upon platinum binding and ethanol addition, ^{31}P NMR spectral studies were carried out. Figure 5A shows the ^{31}P NMR spectrum of sonicated poly(dG-dC)•poly(dG-dC) in 10 mM cacodylate, 1 mM EDTA, pD 7.6. One broad resonance is observed at δ = 4.2 ppm. After modifying the polymer with [(dien)PtCℓ]Cℓ at an r_b of 0.10 the spectrum shown in Figure 5B was recorded. The ethanol concentration was then brought to 40% following which both the CD and NMR spectra were measured. In the inset to Figure 5 are the CD spectra of the platinated polymer before (B) and after (C) the addition of ethanol; the latter is consistent with the induction of Z DNA. The ^{31}P NMR spectrum displayed in Figure 5C shows a new peak centered at $\delta \sim 2.8$ in addition to the 4.2 ppm resonance. The existence and chemical shift of this new peak, which was observed for three different samples, are consistent with the presence of Z DNA as previously reported (3,4,5). The possibility that this new peak is simply due to the presence of platinum on some of the bases was ruled out by the control spectrum of Figure 5B.

Theoretically, the two conformationally inequivalent phosphorus atoms in Z DNA should exhibit two well defined peaks of equal intensity. The relatively low intensity of the downfield resonance observed in our spectra is of interest since the CD results show complete inversion. When attempts were made to increase the intensity of the smaller resonance in the platinated sample by raising the ethanol concentration above 40% a precipitate formed and no spectrum could be obtained. It is possible that, under the conditions of high concentrations ($\sim$1 mM) of

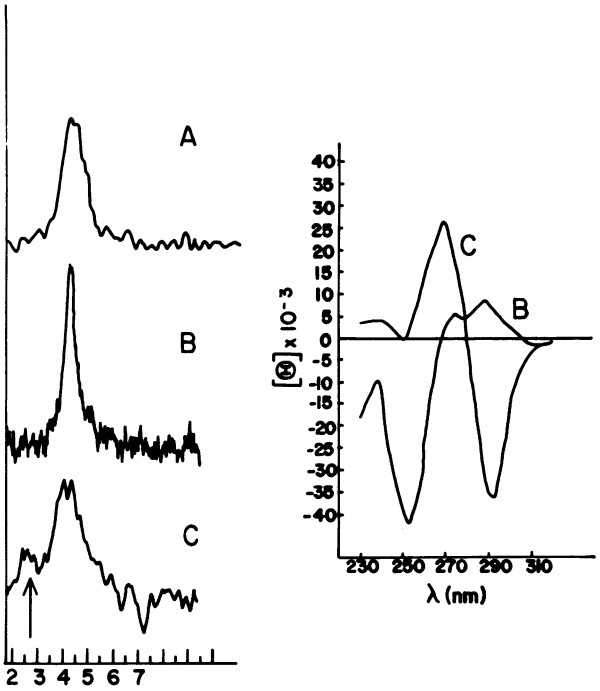

Figure 5. ^{31}P NMR spectra of poly(dG-dC)•poly(dG-dC) measured as described in Materials and Methods. A) spectrum of poly(dG-dC)•poly-(dG-dC) in 10 mM cacodylate, 1 mM Na_2EDTA, pD 7.6; B) spectrum of poly(dG-dC)•poly(dG-dC) in 10 mM cacodylate, 1 mM Na_2EDTA, pD 7.6, modified with [(dien)PtCl]Cl to r_b = 0.10; C) spectrum of sample in B after raising the ethanol concentration to 40%. The arrow designates the resonance characteristic of Z-DNA. On the right are the CD spectra of samples B and C.

platinated poly(dG-dC)•poly(dG-dC) and 30-40% ethanol required for the ^{31}P NMR experiments, aggregation or condensation of the DNA occurs. The CD spectrum of this concentrated solution shows only a large negative absorption at 295 nm and no peak at 260 nm. Upon dilution of the concentrated sample the CD spectrum reverts to that of normal Z DNA. The presence of aggregates, the CD spectral characteristics of which match neither those of ψ DNA (26) or toroidal condensates (24), may explain the broadening and inequivalent intensities observed in the ^{31}P NMR spectra. Aggregation or condensation might convert Z to B DNA, which would contribute to the 4.2 ppm resonance at the expense of that at 2.8 ppm. The presence of both B and Z form DNA has previously been shown to produce intensity inequivalent ^{31}P NMR peaks quite similar to

ours (27). Although we cannot be certain about the state of the DNA in the NMR experiment, the 2.8 ppm resonance is consistent with at least a portion of the DNA molecule being in the Z conformation. Analogous ^{31}P NMR studies with varying NaCl concentrations and no ethanol would be of interest.

The ability of [(dien)PtCl]Cl to promote the B→Z transition of poly(dG-dC)•poly(dG-dC) has both stereochemical and electronic components. Examination of space filling models of the DNA and the platinum reagent showed that the amino protons of diethylenetriamine-platinum(II) covalently bound to N7 of guanine in Z form poly(dG-dC)•poly(dG-dC) are well situated to form hydrogen bonds to a phosphate oxygen atom and to a cytosine O2 atom of the two flanking nucleotides. This hydrogen bonding should contribute substantially to the stabilization of the Z conformation. A second component of stability for the platinum Z DNA interaction is a charge effect. The net charge of +2 on $[(dien)Pt]^{2+}$ covalently bound to the DNA could assist the transition as postulated for the methylation of guanine at N7 (12).

The effect of adding ethanol to samples of poly(dG-dC)•poly(dG-dC) modified with cis-DDP was also studied in order to determine if the antitumor drug could facilitate the B→Z transition. Each of the samples used to obtain the CD spectra shown in Figure 4 was titrated with ethanol up to a concentration of 75% (data not shown) without inducing the B→Z transition. Cis-DDP is known to unwind and shorten closed circular DNA (28,29) and to decrease its reactivity as a substrate for restriction enzymes (29,30). These effects are attributed to a bifunctional interaction of the platinum complex with two adjacent or nearby bases. For Z DNA to form there must be a rotation about the guanine glycosyl bond in order to switch it from an anti to a syn conformation (9). Model studies reveal that cis-DDP can bind in a bifunctional manner to guanine N7 atoms in a d(GCG) sequence. Bifunctional binding of cis-DDP to guanines in d(GAG) sequences has also recently been postulated (31). Such a bifunctional interaction in poly(dG-dC)•poly-(dG-dC) would prevent the rotation of the guanine bases about their glycosyl bonds and prohibit the B→Z transition.

A unique aspect of platinum induced DNA structural changes is that binding to the polymer is largely reversible through treatment with cyanide ion (17,32). Samples of poly(dG-dC)•poly(dG-dC) with r_b values of 0.054 and 0.093 were treated with KCN as described in Materials and

Methods. After thorough dialysis, atomic absorption analysis showed complete removal of platinum. An ethanol concentration of 55% was then determined to be necessary to achieve the B→Z transition (data not shown) demonstrating that the platinum induced structural alteration which facilitates the transition is readily reversed. This reversibility is not readily achieved with other covalently modifying reagents that induce Z DNA such as AAF, which binds to C8 of guanine (10,11), and the methylating agents (12,13). Although $[Co(NH_3)_6]^{3+}$ induces the B→Z transition in poly(dG-dC)•poly(dG-dC) (13), it does so through ionic and not covalent binding.

Is Platinum Binding Enhanced when DNA is in the Z Form? It has been proposed (8), and it is logical to presume, that the increased solvent exposure of the N7 and C8 atoms of guanine in Z DNA facilitate chemical modification at these positions. In order to test this hypothesis the relative binding affinities of [(dien)PtCℓ]Cℓ for B and Z forms of poly(dG-dC)•poly(dG-dC) were measured. Poly(dG-dC)•poly(dG-dC) was placed into the Z form by addition of 60% ethanol or 50 μM $[Co(NH_3)_6]^{3+}$. The inverted CD spectrum, bathochromic shift of the UV maximum, and increased absorbance at longer wavelength characteristic of Z DNA were observed in each case. These samples, as well as B form poly(dG-dC)•poly(dG-dC) in 1 mM sodium phosphate, 5 mM sodium chloride, pH 7.4 were incubated with [(dien)PtCℓ]Cℓ at various r_f values for 24 h. Samples were then dialyzed to remove unbound platinum and the amount of bound platinum was determined by AAS. As a control to account for changes in binding due to solvent and ionic strength effects, a duplicate experiment using poly(dG)•poly(dC), which cannot adopt the Z conformation, was carried out. The data for poly(dG-dC)•poly(dG-dC), shown in Table I, reveal that there is no measurable enhancement of platinum binding to DNA in the Z conformation. Data for the control experiment are not included but the presence of ethanol or hexamminecobalt(III), conditions used to bring about the Z conformation of poly(dG-dC)•poly(dG-dC), did not affect platinum binding to poly(dG)•poly(dC). There was also no difference in the binding of saturating amounts, r_f = 0.5, of [(dien)PtCℓ]Cℓ to B or Z DNA. In both cases, r_b was measured to be the same (Table I). When ethanol was removed by dialysis from the Z DNA sample the CD spectrum reverted to that characteristic of highly platinated poly(dG-dC)•poly(dG-dC), Figure 1. Thus it was not possible to "trap" the DNA in the Z conformation by this strategy.

Table I. Binding of [(dien)PtCℓ]Cℓ to poly(dG-dC)•poly(dG-dC) in the B and Z conformations after 24 hours.[a,b]

r_f	r_b "B"	r_b "Z" Ethanol	r_b "Z" $[Co(NH_3)_6]^{3+}$
0.014	0.013	0.010	0.013
0.027	0.023	0.024	0.026
0.053	0.048	0.042	0.042
0.103	0.088	0.080	0.067
0.50	0.30	0.32	--

[a] Abbreviations: r_f, ratio of added platinum per nucleotide; r_b, measured ratio of bound platinum per nucleotide. [b] Each value has an experimental uncertainty of ±10% associated with it.

The kinetics of binding [(dien)PtCℓ]Cℓ to 100 μM poly(dG-dC)•poly-(dG-dC) in the B and Z conformations were examined. Samples of poly-(dG-dC)•poly(dG-dC) in B form in 1 mM phosphate, 5 mM chloride, Z form in 50 μM $[Co(NH_3)_6]^{3+}$, and Z form in 60% ethanol were incubated at a platinum r_f of 0.11. Aliquots were removed with increasing time, the binding reaction was quenched by raising the chloride concentration to 0.2 M, and the samples were frozen and later dialyzed to remove unbound platinum prior to AAS quantitation of metal binding. The results (Figure 6) indicate that, although the final levels of binding at long incubation times are approximately equal, the rates of initial binding are different in the order B DNA (5 mM $Cℓ^-$, 1 mM phosphate) > Z DNA (60% ethanol) > Z DNA ($[Co(NH_3)_6]^{3+}$, 50 μM). We attribute this ordering to the diminished rate of platinum hydrolysis in the media required to induce the Z form of DNA, since the rate determining step of platinum binding to DNA is known to involve replacement of chloride ion by water (33). The influence of the DNA structure on the kinetics of binding, assuming there is one, would therefore be masked by the effect of the solvent medium on the hydrolysis of the Pt-Cℓ bond. In order to circumvent the rate limiting hydrolysis step, the aquated form of the platinum complex was prepared and allowed to react for various times with poly(dG-dC)•poly(dG-dC) in the B and Z forms. The resulting binding kinetics were very fast for $[(dien)Pt(H_2O)]^{2+}$ compared to those of $[(dien)PtCℓ]^+$, however, and it was not possible to determine the influence of the polymer conformation on the reaction rate.

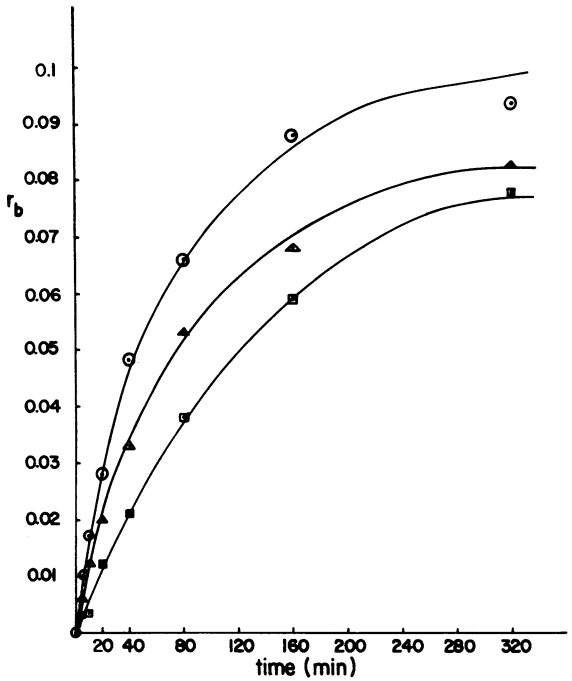

Figure 6. The kinetics of binding [(dien)PtCℓ]Cℓ to poly(dG-dC)•poly-(dG-dC) at 37° in 1 mM sodium phosphate, 5 mM NaCℓ, pH 7.4 buffer (⊙), in the same buffer with ethanol added to a concentration of 60% (△), and in the presence of 50 μM $[Co(NH_3)_6]Cℓ_3$ (⊡). The nucleotide concentration was 100 μM and the initial ratio of [(dien)PtCℓ]Cℓ to nucleotide, r_f, was 0.11.

Conclusions. The major finding is that [(dien)PtCℓ]Cℓ facilitates the B→Z transition in poly(dG-dC)•poly(dG-dC) whereas the antitumor drug cis-diamminedichloroplatinum(II) blocks it. The chelating tridentate diethylenetriamine ligand requires [(dien)PtCℓ]Cℓ to react with the biopolymer in a monofunctional mode. The tightly bound, positively charged complex stabilizes the Z form through electronic and specific hydrogen bonding interactions. The antitumor drug, which is known to react with DNA in a bifunctional manner, prevents the conformational changes necessary to induce the B→Z transition. The binding of [(dien)PtCℓ]Cℓ to poly(dG-dC)•poly(dG-dC) and the concomitant B→Z structural changes are readily reversible. Finally, we have shown that there is no difference in the metal binding affinity to DNA in the B and Z conformations.

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